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# Epithelioid Cell: A New Opinion on Its Nature, Parentage, Histogenesis, Cytomorphogenesis, Morphofunctional Potency, Role in Pathogenesis and Morphogenesis of Tuberculous Process

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## 1. Introduction

It is known that during an embryogenesis form a well-defined for each type of organism the number of cell types, each of which has its own specific morphophysiological characteristics. Some cells operate only at certain stages of embryonic development and then disappear as a result of apoptotic death, while others are unique for adult organism. However, in researching of granulomas formed in some granulomatous diseases have been described cells are not found in a healthy organism. To such cells referred «basophilic histiocytes» in rheumatic disease, Mikulich cells in scleroma, epithelioid cells (ECs), forming ECs granulomas in a number of infectious, including tuberculosis, as well as some allergic and autoimmune diseases. It was shown that ECs forming in nidus of the inflammation in granulomatous diseases of different etiology. Suppose that ECs do not enter number of differentiated cell-like types neither embryonic not adult organism; they occur only at particular pathological statuses and forming ECs-granulomas. This granulomas determine clinicomorphologic essence of many granulomatous diseases in man. Moreover, the ECs-granulomas form in different groups animals, relating to different branches of "phylogenetic tree". Thus, ECs formation in the nidus of inflammation can be related to one of most ancient mechanisms of cell-like response on imbalance of the "antigenic-structural" homeostasis in organism. The concept of ECs origin from cells of macrophages (Mphs) family till now is considered conventional, which some theoretical fundamentals were placed in workers of Ashoff L. (1924) and Maksimov A. (1926). Affirms that ECs transform from Mphs located in the nidus where the pathological process flows past and under some conditions - directly from monocytes of a blood. This concept is based on the hypothesis that in a basis of the differentiation resulting in to derivation ECs from Mphs in reply to particular pathogenic stimulus lie the changes of genic activity and that in a basis of this transformation resulting in to formation ECs lie epigenetic changes, and the phenomenon can be considered as "intra-tissue transdetermination" (Shvemberger, 1976). Important thus to mark that till now is not obtained sufficiently convince facts touching not only the

mechanisms of transformation of Mphs into ECs, but also process of series transformation of Mphs into ECs. It is explained to that indicated the concept and hypothesis based mainly on the results of classic morphological researches, in which as well as in many modern morphological works, registered only the fact of appearance of ECs in populations of cells of macrophage type without the analysis of the transition forms from Mphs to ECs. It is necessary thus to underline that in none of works dedicated search of ECs metastructure, is not obtained of enough convincing and indisputable evidences of existence of the legible transition forms between Mphs and ECs. Moreover, there are no convincing facts which would testify that differentiated Mphs can undergo dedifferentiation that is switch on in the process being a basis of possible conversion cell-like phenotype. At usage of cell-like technologies in learning ECs cytomorphogenesis was obtained the in essence new facts, which have forced to refuse the concept of origin of ECs from Mphs (Arkhipov, 1995). The application of different cell-like technologies (cultivation in vitro, explantation of cells of granulomas in cultures etc.) allows to place that among peritoneal cells (PCs), mononuclear blood cells and bone marrow exist low-differentiated cells - ECs-precursors (pre-ECs), distinguishing from cells of macrophage series on number of cytomorphologic identifier, registered in vitro. Obtained data allow to confirm the hypothesis that exist unipotent precursors ECs (pre-pre-ECs), which differentiate only into ECs at defined conditions combined in the nidus of chronic inflammation (Arkhipov, 1996). All stages of differentiation of pre-ECs into mature cells of epithelioid type possessing about proliferative activity are defined. On the basis of the obtained data lay down the new conception of origin and differentiation of ECs (Arkhipov, 1997). Is was shown that of ECs-germ forming in norm quantitatively restricted population of low-differentiated monocytoid blood cells being committed cells precursors of ECs. In cronic inflammation the pool of pre-ECs in organism increases. By cytomorphologic characteristics pre-ECs were referred to the class of reticular cells. To the present time obtained the new experiment data indicating the existence a genetic determinancy of a datum basal level ECs reactivity concerning different inductors of an inflammation. Set a question on correlation between function and phenotypic variation of ECs. Data obtained directing that the morphogenesis of ECs granulomas might determine by the several factors: initial genetically determinate level of a pool pre-pre-ECs, inflow pre-ECs, committed into ECs, trended to differentiation in the nidus of inflammation, and also intensity of processes of their proliferation and differentiation. The data obtained allow in a new fashion to formulate a hypothesis about a probable origin and early stages of a histogenesis ECs, namely that ECs might the descendants of mesenchymal stem cells of a bone marrow parentage, out of which differentiate some stromal cells of organism. The clearing up of early stages of ECs histogenesis will allow to answer the question not only about biological essence of ECs forming in different chronic granulomatous processes, including tuberculosis, but also more precisely to spot their function assignment in an organism in pathology.

## **2. The morphofunctional characteristics of epithelioid cells, generated in the foci of granulomatous inflammation in different granulomatous diseases, including tuberculosis**

Morphological manifestation of many granulomatous diseases is forming epithelioid-cell-like granulomas (Alamelu, 2004; Epstein, 1991; Kojima, 1996; Shkurupy, 2007; Strukov & Kaufman, 1989). ECs will be derivated in the centers of a chronic inflammation in

granulomatous diseases of different etiology. ECs-granulomas or the separate clumps of ECs will be derivated in many infectious diseases, including in tuberculosis (Malik et al., 1999; Russell et al., 2009; Shkurupy, 2007), some rheumatic (Chadarevian et al., 1993), autoimmune (Ren, 1992), lymphoproliferative diseases (Takeshita et al., 1993), histiocytoses (Goerdts et al., 1993), in development of tumors of a different histogenesis (McCartney, 1995), granulomatous diseases of a unknown etiology (Kaneishi et al., 1995; Tozman, 1991), hit in an organism of salts of some metals (Haley et al., 1994; Kelly, 1993), foreign bodies (McCarthy et al., 1993), allergens of a different nature (Yamanaka et al., 1994). The issue of granulomatous process largely depends on dynamics of epithelioid-cell-like cytomorphosis in the centers of an inflammation that apparently is specified by morphofunctional features of ECs, permitting by it to fulfill simultaneously functions of boundary conditions (Hasegawa et al., 1994; Noga et al., 1989), exocellular damage of pathogen agents by products of secretion (Baba et al., 1992; Myatt et al., 1994; Tanaka et al., 1996), modulation of Mphs function activity (Mariano, 1995; Miyazaki et al., 1992; Shigenaga et al., 1995), lymphocytes (Abe et al., 1990; Chensue et al., 1992) and fibroblasts (Allen, 1991; Limper et al., 1994). Now is generally acknowledged that ECs are transformed from Mphs in an organ or tissue, where pathological granulomatous process is developed (Dastur et al., 1995; Moraes & Moraes, 1993; Russell, 2009; Takahashi et al., 1994), and under certain conditions - from monocytes of a blood (Facchetti et al., 1989; Williams & Williams, 1983). When is spoken about transformation of monocytes into ECs it is supposed that the monocytes have equal potencies to differentiation into Mphs and ECs (De Vos et al., 1990; Kowalewsky, 1976; Noble et al., 1989). In the concept of a parentage of ECs from Mphs to different subpopulations of lymphocytes tapped the role of controllers of transformation Mphs into ECs, and Mphs appear in a role of acceptors of differentiating signals of lymphocytes (Haley et al., 1994; Horvath et al., 1993). Thus, the accumulation ECs in the center of granulomatous inflammation is reduced to stochastic (on probability of an induction) and continuous transformation Mphs into ECs (Haley et al., 1994; Okabe, 1994). Three types of ECs are now defined: plasmacyte-like, vesicula-like and fibroblast-like (Epstein, 1991; Horiuchi & Masuzawa, 1995; Rasmussen & Petersen, 1993). T. Williams and W. Williams (1983) distinguished two types of ECs: plasmacyte-like, which on structure of a nuclei and development of a granular cytoplasmic reticulum remind plasma cells or plasmacyte (type A), and vesicula-like (type B). Under the data of these researchers ECs such as type A more often meet at early stages of granulomagenesis, ECs such as type B - in more late period. Under the data of Shkurupy and colleagues (Shkurupy et al., 1993), on the contrary, the cells such as type B meet at early stages of generation of ECs-like granulomas. Consider that features of structures of vesicula-like ECs testifies to an expressiveness of their synthetic and secretory function (Shkurupy, 2007; Turk, 1989). It is fixed that the vesicles of ECs advance to cell plasmalemma with the subsequent exhaust of their contents on exocellular medium (Baba et al., 1992). It is exhibited that bactericidal and also the secretory activity in ECs-granulomas is more expressed than in mature macrophage granulomas (Abe et al., 1990; Tanaka et al., 1996). In ECs in the field of Golgi lamellar complex are taped not only zonated, but also sleek vesicles with dense center, and also great many (more than 100) large granulas with diameters up to 340 nm and with finegranular matrix more light than in macrophage granulas, sometimes with perigranular halo (Rhee et al., 1979; Samtsov & Shiliaeva, 1990). The number of lysosomes in ECs enlarged in comparison with their number

in monocytes and Mphs. Some types of such structures are detected: 1) homogeneous lysosomal; 2) with myelinic bodies; 3) having frame multivesicular bodies; 4) with major irregular crystalline inserts. The cell-like center in the majority of ECs more volumetric than in mature Mphs is marked. Thus, ascending density of filaments in cytoplasm, which have radial orientation and diameter 5-6 nm or about 10 nm is revealed (Epstein, 1991; Horiuchi & Masuzawa, 1995; Turk, 1989). Under the data Rhee (1979) secondary lysosomes and macrophage granula in such cells are not taped. In ECs such as type B the specific composition of enzymes (acidic phosphatase,  $\beta$ -galactosidase, nonspecific esterase, peroxidase, lysozyme, angiotensin converting enzyme), and also factors affecting the activity of fibroblasts and them collagengenerating function are detected (Allen, 1991; Inuzuka et al., 1994; Limper et al., 1994; Miyazaki et al., 1992; Turk, 1980). It is fixed that the angiotensin converting enzyme can brake migration Mphs, that is play a role of the factor inhibiting migration of Mphs, that is important for formation of cell-like aggregate in granulomagenerating process (Mariano, 1995; Williams & Williams, 1983;). At usage enzyme-linked immunoassay methods it is exhibited that ECs produce IL-1, IL-2, IL-4, IL-6, TNF- $\alpha$  (tumors necrosis factor), multifunctional growth factor TGF- $\beta$  (Limper et al., 1994; Myatt et al., 1994; Toossi et al., 1995). These data specify that ECs play important regulatory function in formation of granulomas and in pathogenesis of granulomatous diseases. In a sarcoidosis on a surface of ECs happens expression HLA-DR (Ia) and HLA-DQ of antigenes (Hoffmann-Fezer et al., 1992). Apparently, it promotes immunological interaction of these cells with T-lymphocytes. The majority of investigators registered that ECs have lower phagocytic activity in comparison with Mphs. In cytoplasm of ECs the lipids, which are surveyed as oddments of the killed and digested micro-organisms, also crystalloid frames, asteroid bodies, formation such as phagosomes and multivesicular bodies, are detected (Horiuchi & Masuzawa, 1995; Navarro et al., 1992). It is necessary to underline that there are contradictory enough data about ability of ECs to phagocytic response. One investigators register absence of phagocytosis for ECs (Momotani et al., 1993; Velge et al., 1994) and other specify existence weak phagocytic activity of ECs, and also on boundedness of a population of ECs, in which the objects of phagocytosis are registered (Desportes-Livage et al., 1996; Hoop et al., 1994). At the same time bactericidal and secretory activity of ECs is expressed more strongly, than for Mphs (Kumar et al., 1989; Turk, 1980; 1989). The inconsistency of the data about ability ECs to an phagocytosis is to some extent explained by the concept "divergent differentiation" of monocytic blood cells, migrating to the center of inflammation, caused by microorganisms. According to this concept it is supposed that one of monocytes specialize on an phagocytosis and other - losing phagocytic potency, only pinocyte particular yields of decay (cytophagous material), and are transformed into ECs (Kowalewsky, 1976). Under the data Baba and colleagues the organizations cytoskeleton of ECs in the center of an inflammation more compatible to cytoskeleton characteristic of epithelial cell than to cytoskeleton of active and movable Mphs (Baba et al., 1992). The three-dimensional metastructure of ECs in usage of methods of prompt freezing, penetrating etching and freeze-substitution was studied (QF-FS-method). The granulomas were caused in rats by injections of muramyl dipeptid. It is exhibited that the dense webs of intermediate filaments, bound with cores, mitochondrions and other organellas, are supervised everywhere in cytoplasm of ECs. Some fascicles of actinic filaments were posed in filopodiums below than membranes of the cells. Exact interdigital tripping of membranes of



cells between interfacing ECs were clearly demonstrated by QF-FS- method. The so-called coated pits (zonated fossas) in a basis of interdigital filopodiums are identified. The characteristic indication of ECs is their aggregation with formation tight interdigital tripings as a fastener "lightning", which, apparently, can have the important value in differentiation of these cells (Baba et al., 1992; Noga et al., 1989). It is marked that on early terms of generating of granulomas the indicated frames miss. Probably, they ensure particular "localizing" and biochemical barrier for different pathogen agents, and also fastness in relation to proteolytic enzymes. At the present time the monoclonal antibodies IHY-1, which react only with ECs in sarcoid granulomas, are obtained (Ishioka et al., 1990; Ishioka & Yamakido, 1990). These antibodies did not react with erythrocytes, lymphocytes, monocytes, alveolar Mphs, and also with macrophage derivatives - cells of cultures U-973 and KG-1. These monoclonal antibodies also reacted with ECs in granulomas of lymphatic clusters in tuberculous persons (Ishioka et al., 1990). Under the data of the different contributors already it is possible to present immunological phenotype of mature ECs. In the whole series of different operations by the immunological methods identified following antigenic markers on cells with morphological phenotype ECs: RFD-9, IHY-1, CD1, CD4, CD11, CD14, CD25, CD31, CD36, HLA-DR, HLA-DQ, OKDR, MAC-387, OM2, 25F9, KiM1P, ICAM-1 (a5, b2), LFA-3 (Boehncke et al., 1993; Cerio et al., 1990; Hoffmann-Fezer et al., 1992; Ruco et al., 1992; Spiteri et al., 1989). However, now from known markers only antibodies named RFD-9 (Munro et al., 1987) and IHY-1 (Ishioka et al., 1990) allow precisely enough to differentiate ECs from other classes of cells. The functions of proteins, to which the indicated antibodies yet are bound did not fix. It should also be noted that many data obtained by different researchers, should be treated fairly critically and carefully, because the first thing necessary for the immunological identification of cells - is an accurate identification of morphofunctional types of cells. At the same time in many operations dedicated an indicated problem, will be utilized only a few morphological, is legible not of particular tests of identification of ECs, which are very subjective and can give errors at definition phenotype of ECs. Moreover, there are some terminological indeterminacies. For example, often use the term "epithelioid Mphs", «epithelioid histiocytes" (Aguiar-Passeti et al., 1997; Orrell et al., 1992; Westwood et al., 1995). At the same time morphologically is not improved, what cells are available in view of typical ECs or activated Mphs, which having any indications that are the characteristic for ECs. It is necessary still pay attention and to that fact that many cell-like structures or products (antigenes, receptors, cytokins) already are detected, which are characteristic not only for ECs or Mphs, but also for cells of other types, for example, dendritic cells, lymphocytes, cells of an endothelium and even of erythrocytes. So, for example, antigenes HLA-DR (Ia) and the molecules of a cell-like adhesion (ICAM-1) are detected not only on Mphs and ECs, but also in cells of an endothelium, dendritic cells, and also interdigitate reticular cells of lymphoid organs and tissues (Giotaki et al., 1992). The protein S100 is detected in ECs, Mphs, dendritic cells, cells of an endothelium, in all types of a macroglia, Schwann cells, and also in small amount in neurones, adipocytes, chondrocytes and lymphocytes (Hachitanda et al., 1990; Momotani et al., 1990; Momotani et al., 1993). Differentiation antigen CD68, characteristic for Mphs, were determined not only in ECs, but also in lymphoblastic leukosis cells, and also in spindle-shaped tumoral cells, which are included in cell-like composition of a fibroxanthoma (Horny et al., 1993; Longacre et al., 1993; Kodelja & Goerd, 1994). The antigen CD11c is taped on cytoplasmic membranes of

ECs, Mphs and polymorphonuclear leucocytes. Angiotensin converting enzyme is present in Mphs, ECs and cells of an endothelium (Abe et al., 1990; Allen, 1991; Inuzuka et al., 1994), collagenase - in Mphs, ECs, huge multinuclear cells, endothelial cells and fibroblasts (Santavirta et al., 1993). The antigen CD25 is detected on ECs, activated T-lymphocytes and NK-cells; CD1 - on ECs, thymocytes and arborescent cells of a skin (Langerhans cells); CR3 - on Mphs, ECs, T-lymphocytes, NK-cells and granulocytes (Horny et al., 1993; Kodelja & Goerdt, 1994). Thus, many facts obtained by the different researchers in study the morphofunctional characteristics of ECs, are contradictory enough and are not completely stacked in the concept of a parentage ECs from Mphs.

### **3. Criticism of the modern concept of origin of epithelioid cells from macrophages and other cells of the system of mononuclear phagocytes**

It is now considered conventional representations that the different forms or types of ECs are derivative of Mphs and will be derivated in the center of granulomatous inflammation, for example in tuberculosis, as a result of transformation Mphs or monocytes (precursors of Mphs) into ECs [Cipriano et al., 2003; Turner et al., 2003]. This concept was formed many decades back on the basis of numerous morphological examinations of tubercular process, and also other granulomatous diseases of infectious and noninfectious etiology. The basis of this concept is that fact that the formation of ECs happens among Mphs in forming granulomas. Thus in generating ECs-like granulomas, for example in tuberculosis, the ascending of amount of ECs in granulomas interface to relative decrease in them Mphs. Thus, was quite logical to assume that ECs will be derivated from Mphs. The analysis of the literature, in which are mentioned ECs, generated in granulomas in tuberculosis or in granulomatous processes of other infectious etiology, testifies that till now was not conducted of any examinations, in which the convincing data confirming the fact of transformation Mphs into ECs would represent. The majority of the modern contributors, surveying problems touching ECs, and speaking about a parentage of ECs, is written with such phrases: «as is known ECs will be derivated from Mphs», «it is considered that ECs are derivative of Mphs and monocytes». However, at the best they refer to any old operations, in which was not given the convincing proofs indicative of transformation Mphs into ECs. In the scientific work of Sutton and Weiss (1966) was exhibited that in culture of monocytes of a blood of a chicken two types of cells - Mphs and ECs are formed (Sutton & Weiss, 1966). However, not any proofs of transformation Mphs into ECs then are represented. The similar data were obtained also by other investigators in other pilot models indicative of that in cultures of monocytes of a blood can be formed Mphs, ECs, and also huge multinuclear cells of two types - "foreign bodies" and cells, similar Langerhans cells in tubercular granulomatous inflammation (Levis, 1925; Levis & Levis, 1926; Nakagavara et al, 1981; Sutton, 1967; Zuckerman et al., 1979). However, and in these works the speech went formation of the different cell-like forms in culture of monocytes, the heterogeneity of which cell-like composition now does not call doubts. In the scientific work of Pulford and Souhami (1980) was exhibited that in cultures of cells enriched with Kupffer cells, the formation ECs is recorded (Pulford & Souhami, 1980). However, quality of a fractionating and identification of Kupffer cells, having been available in the 80-s years, does not allow to speak that among explanted in cultures of the Kupffer cells there were also other undifferentiated types of cells. In the review Spector and Lykke (1983) have written that «mononuclear phagocyte origin is not in doubt, but there remains controversy over the

mechanisms by which epithelioid cells are formed, and in particular the role of cell-mediated immunity» (Spector & Lykke, 1983). At the same time they refer to series of operations (Adams, 1974; Turk & Narayan, 1982), in which be not represented of the data proving a position about an opportunity of transformation Mphs into ECs. In the scientific work Rhee et al. (1979) an issue about differentiation of monocytes in Mphs, and then in ECs and multinuclear cells, is regarded. However, of any direct evidences indicative about differentiation Mphs into ECs, is not resulted (Rhee et al., 1979). Only some cytomorphologic and cytochemical features of monocytes, Mphs and ECs, are surveyed, which do not allow uniquely to conclude that ECs will be derivated from Mphs, instead of from any variety of a monocyte or monocytoïd form of cells. But they links to other contributors (Adams, 1974; Papadimitriou and Spector, 1971), in which operations nor represented one valued and stipulated facts proving that ECs will be derivated as a result of transformation Mphs. In the scientific work Mariano et al. (2003) the data indicative that peritoneal Mphs, cultured in medium, containing heightened concentration IL-4, gain in their judgement, some morphological features of ECs, characteristic for them in culture, are obtained (Mariano et al., 2003). However, on our view, introduced in this operation of a photo and other data, testify only about major phenotypic variability of Mphs, as in operations of other writers this phenomenon was scored earlier. In the scientific work Stanton et al. (2003) the attempt was made to construct the scheme of differentiation ECs from resident Mphs in lung in tuberculosis at usage of an estimation of several enzymes in cells, including angiotensin converting enzyme (Stanton et al., 2003). However, as writers of this operation, obtained by them, the data recognize do not allow to solve the problem whence take Mphs - precursors ECs. In the logic build-ups they based on a parentage ECs from cells of system of mononuclear phagocytes. Thus, it is possible to make the inference that the concept of a parentage ECs from Mphs and monocytes, as precursors of Mphs, was formed still in 60-80-th years of the last century under effect of operations, dedicated effects of transformation of monocytes into Mphs, ECs and huge multinuclear cells in vitro, started Maximow (1925), Levis (1925), and also other "«more late" classical morphological examinations of granulomatous inflammatory processes (Levis, 1925; Maximow, 1925). Actually this concept is grounded on a position about existence among "monocytes" only one populations of cells - precursors of Mphs. This circumstance requires treating a problem about a parentage ECs from modern positions. If ECs are differentiated from Mphs, why nobody has described of the legible transition shapes of these cells? If ECs gain any new functions, how there is their becoming? If ECs are differentiated from Mphs or monocytes, whether that can on any measure be spotted phenotype of Mphs or monocytes, which become on a trajectory differentiation in ECs? As the answers to these problems in the scientific literature is not present, it was represented very important and interesting to receive detailed exposition of all stages differentiation of ECs from Mphs in vitro.

#### **4. The results of own experimental researches, indicative that epithelioid cells will be derivated not from macrophages and monocytes (the precursor of macrophages), but from monocytoïd cells, committed in a epithelioid-cell-like direction of differentiation**

In the series of operations it is exhibited that in cultures of tissues and cells, containing Mphs or monocytes, ECs are taped 2-3 weeks after beginning of cultivation (Arai et al., 1999;



Kodelja & Goerdt, 1994; Rhee et al., 1979). We originally attempted to reveal by morphological criteria a subpopulation of Mphs, possessing particular potencies to differentiation into ECs. It is fixed that for 7 days of cultivating in cultures of PCs of intact mice line BALB/c there are large cells of an epithelioid type distinguished from cells of macrophage type on a lot of morphological indications. In standard colouring by azure-eosine the cytoplasm of these cells gained an acyanotic grey - blue tone, and the nuclei (as against macrophage cells of a type with intensive colouring of nuclei) in light pink colour and had a chromatin with the weakly expressed reticulate structure were coloured. These cells were also expressed by major sizes of nuclei of the oval shape and cytoplasm, which contours, as a rule, gained the polygon shape, and also major nucleolus. On the first investigation phase it was necessary to answer one more question. Whether it is possible to survey cells of an epithelioid type formatived in cultures of PCs of mice, as typical ECs, formatived in the centers of chronic granulomatous inflammation in vivo? It is known that the hypodermic introduction of a Freund's complete adjuvant results in development of granulomas, containing in the composition the ECs. It is known also that the intraperitoneal or intravenous introduction of mycobacteria of BCG vaccine can result in inductions chronic granulomatous inflammation with generating of ECs-like granulomas in a liver (and other organs) of mice (Orrell et al., 1992; Shkurupy, 2007). Thus, it is possible to survey vaccine BCG and Freund's complete adjuvant, containing in the composition heat-killed micobacteria of a tuberculosis, as particular inductors epithelioid-cell-like forming in the center of granulomatous inflammation. Starting from these positions, it was possible to

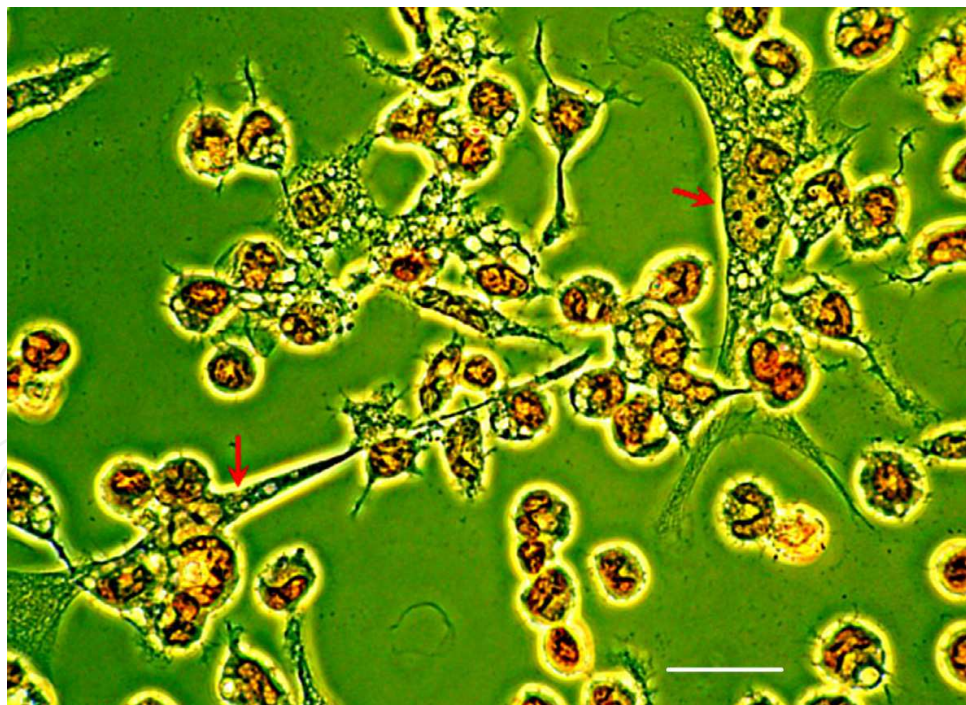


Fig. 1. Culture of PCs of mice of line BALB/c, beforehand stimulated in vivo (7 days prior to an explantation of cells) by Freund's complete adjuvant (intraperitoneal introduction 50 % of emulsion in physiological saline solution), 7 days cultivated. Plasmacytoid EC (right) and fibroblast-like ECs (left) - are marked by arrows. In Mphs and ECs the builders of an adjuvant are visible on the basis of a vaseline oil. A method of colour interferential contrast (cytomorphologic analysis in yellow-green area of a spectrum). Scale bar: 25  $\mu$ m.

expect that the intraperitoneal introduction of the indicated inducers can give in a stimulation of processes of formation ECs in cultures of PCs.

The legitimacy of the expressed guess was confirmed in following experiment. In 7 days after intraperitoneal introduction into mice of line BALB/c Freund's complete adjuvant, cells of peritoneal transsudate explanted in cultures and cultivated in vitro within 5-7 days. It is fixed that in 5-7 days of cultivating in cultures of PCs, stimulated by Freund's complete adjuvant, the amount of cells of an epithelioid type will increase in comparison with the control at 7-10 of time. Moreover, in such stimulated cultures were shaped not only small epithelioid-cell-like clusters, consisting from 3-5 ECs, but also major monolayer layers consisting of fitting closely to each other mature cells of an epithelioid type, morphologically similar to layers of typical epithelial cells that cultivating in vitro. On morphology by using the method colour interferential contrast (Arkhipov, 2002) was chosen 3 forms of ECs formatived in cultures of PCs: fibroblast-like, plasmacytoid and vesicul-like ECs (fig. 1). The cells of a type plasmacytoid forms always dominated. The intravital observations (in microchambers) by process of forming of epithelioid-cell-like clusters and layers have allowed to make the conclusion that the gain value of cells in epithelioid-cell-like clusters and layers is carried out by direct division of ECs (as a result of processes of an endomitosis and amitosis), taking place mainly on a periphery of clusters or cell-like layers, with subsequent cytotomy (fig. 2).

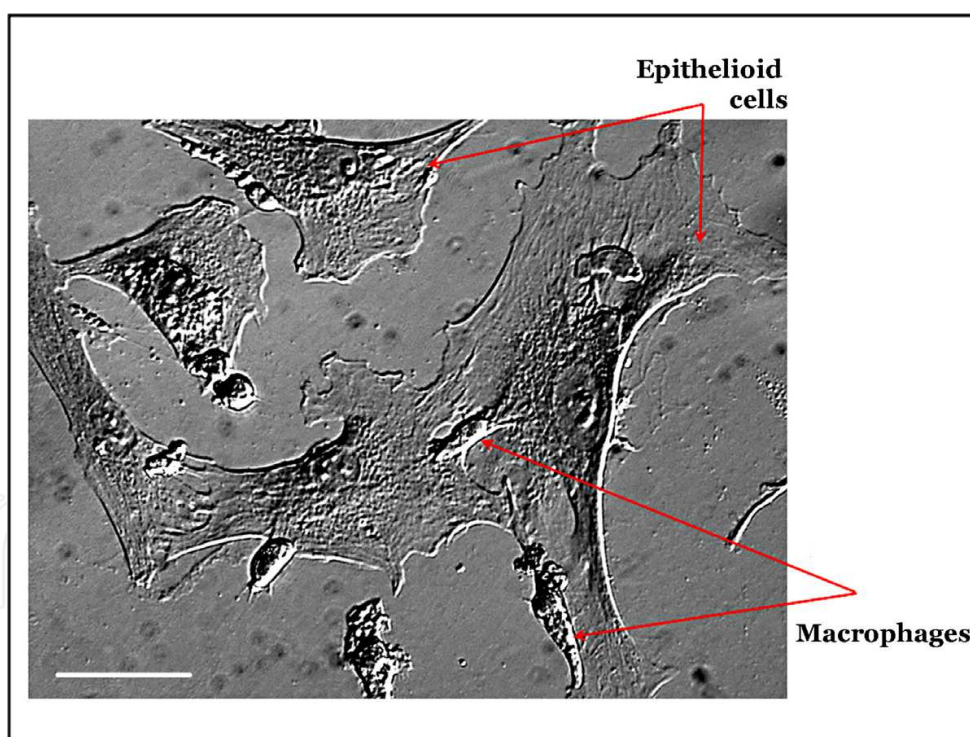


Fig. 2. Epithelioid-cell-like cluster, formed in culture of PCs of mice of line BALB/c, beforehand stimulated in vivo (7 days prior to an explantation of cells) by Freund's complete adjuvant (intraperitoneal introduction 50 % of emulsion in physiological saline solution), 10 days of cultivating. A method of differential interferential contrast. Scale bar: 30  $\mu$ m.

Thus as a result of disproportionate cytotomy from one EC some "affiliated" (or "child") cells of the smaller sizes formatived mainly of cones of cytoplasm growth can be formed at



once. The majority ECs, taking place in clusters, gain the polygon shape (fig. 3). Thus, epithelioid-cell-like clusters and the layers formed in cultures of PCs, gained features of typical cultures of epithelial cells formed in a monolayer (on morphology, locating in a monolayer and type of cell-like body growth).

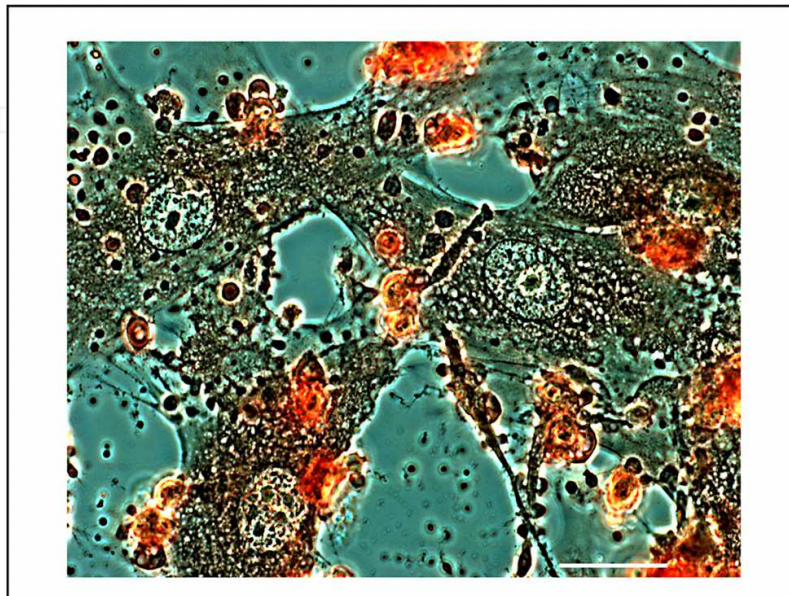


Fig. 3. Epithelioid-cell-like cluster, formed in culture of PCs of mice of line BALB/c, beforehand stimulated in vivo (7 days prior to an explantation of cells) by Freund's complete adjuvant (intraperitoneal introduction 50 % of emulsion in physiological saline solution), 14 days of cultivating. Among ECs are visible numerous apoptotic bodies of macrophages; by red colour are painted the apoptotic changed macrophages. A method of colour interferential contrast. Scale bar: 40  $\mu\text{m}$ .

Most visually, cytomorphologic difference between Mphs and ECs, generated in culture of PCs, are visible at usage for image analysis of cells of method of color coding of the numeric images of cells. At usage of the method color coding on a brightness of image, consisting in assignment to pixels with particular luminosity of particular colour or a monochrome color tone, contrasting with by other contiguous "cluster" of brightness gradation, the new colour image of mixed culture Mphs and ECs was received, which grows out of colour code translation of the starting image of cells. At polychromatic pseudo-colouring Mphs and ECs, obtained computer transformation of the starting image, it is visible as far as are essential cytomorphologic difference of Mphs and ECs, defining some kind of different «color phenotypes» describing them cytomorphologic differences (fig. 4).

Based on these data were formulated two working hypotheses appear in cultures of peritoneal cells clusters of ECs. According to the first assumption mature macrophage cells, transforming into ECs, can gain proliferative activity. However, it contradicts modern representations that in process of differentiation the proliferative activity of cells of monocytic-macrophagic histogenesis is considerably reduced or is completely lost. Thus, if to proceed from the conventional concept of a parentage ECs from Mphs, then it is necessary to enter more still as a minimum two assumption that mature Mphs can undergo dedifferentiation, start to proliferate, and in dedifferentiated Mphs or undifferentiated

monocytes (precursors Mphs) the new genetic program differentiation, starting purely others, though and in something similar with Mphs, biochemical metabolic pathways of synthesis of new substances resulting in to generation epithelioid-cell-like phenotype, should somehow join.

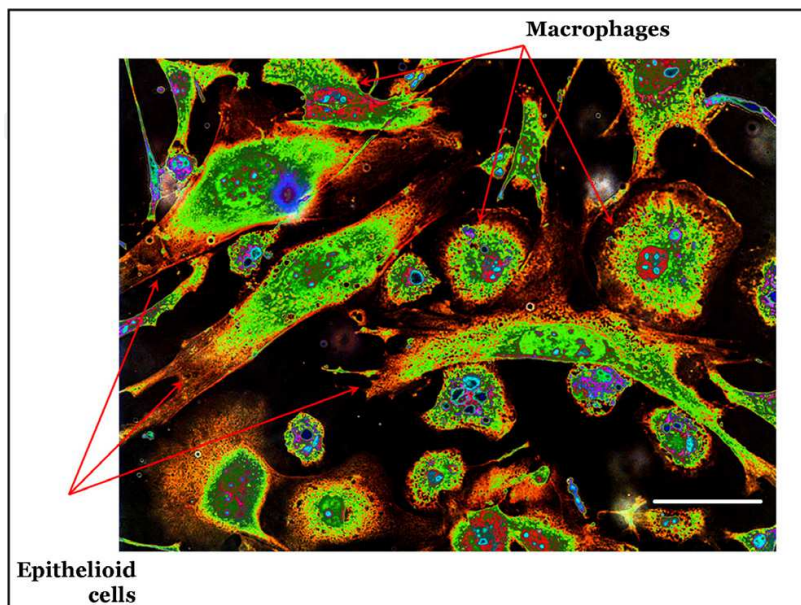


Fig. 4. Culture of PCs of mice of line BALB/c, beforehand stimulated in vivo (7 days prior to an explantation of cells) by Freund's complete adjuvant (intraperitoneal introduction 50 % of emulsion in physiological saline solution), 10 days of cultivating. Mphs and ECs are marked by by arrows. Colouring by azure-II-eosine. A method of color coding of the numeric image (color coding of contrasting color hues on 20 gradation of brightness). Scale bar: 40  $\mu$ m.

According to the second hypothesis the mechanism of formation of epithelioid-cell-like clusters in culture of cells could be stipulated by existence some kind of chain reactions (effect "of an impinging domino"), when one mature differentiated EC stimulates processes of an induction neighboring Mphs, including mechanisms differentiation of these cells in an epithelioid-cell-like direction. This hypothesis was discarded after the first experiments in vitro, as in composition of epithelioid-cell-like clusters was not detected of any cell, which could be surveyed as the transition form from Mphs to a cell of an epithelioid type. As a whole cytomorphologic analysis of cultures of PCs beforehand stimulated by Freund's complete adjuvant has shown that on 5-7 days cultivating is possible in cultures to differentiated some types of cells: 1) the cells of a macrophage type, absolute majority from which phagocytized granulas of a zymosan (GZ); 2) ECs (not phagocyting); 3) fibroblast-like cells (not phagocyting); 4) huge multinuclear cells of a macrophage type (phagocyting), huge multinuclear cells of an epithelioid type - similar cells Langhance (not phagocyting), multinuclear cells of a mixed type derivated as a result of fusion Mphs and ECs - "symplast". By the way opportunity of formation "symplasts" from Mphs and ECs allows to explain - how objects of an phagocytosis (for example, the micobacteria of a tuberculosis) can turn out in cytoplasm of ECs that is not as a result of natural phagocytic activity, but in result mergings with Mphs, which already have imbibed micobacteria. At the total analysis of all cytological preparations of cell-like cultures of PCs, obtained in different experiments in vitro, was not detected of cells, which could be referred to morphological indications



simultaneously both to Mphs and to ECs that is to the transition forms of cells from Mphs to ECs. As ECs did not phagocytize the granulas of a zymosan, is not time-dependent additions of granulas into cultures, was apparent that the cells-precursors of ECs and their transition forms have no phagocytic activity. GZ were detected only in symplasts, generated as a result of fusion Mphs and ECs. During examination in cultures of intact PCs and stimulated by Freund's complete adjuvant were detected rather large monocyte-like cells, distinguished from typical monocytes, which on the sizes, colouring, morphology of nuclei (and their colouring), shape of spreading it was possible to refer to the transition shapes ECs of a different degree of maturity. These cells differed from typical ECs of epithelioid-cell-like clusters, included in composition, and in ECs-layers, only by small sizes. Conditionally these cells were termed "juvenile" or "young" ECs. Starting from these tentative datas, the new alternate hypothesis epithelioid-cell-like generating in vitro was formulated, which essence consists that ECs in cultures of cells are formed not from Mphs, but of any low-differentiate mononuclear cells – precursors of ECs – the cells of monocytoïd type with some cytomorphologic features, distinguishing them from typical monocytes.

### **5. The characteristic (morphofunctional and cytochemical) of epithelioid cells and its cells-precursors (pre-ECs), contained in abdominal cavity after induction of an experimental peritonitis (intraperitoneal introduction by Freund's complete adjuvant) – Development of cytomorphological criteria of identification of epithelioid cells and pre-ECs in vitro**

For targeted searching for low-differentiate mononuclear cells - precursors of ECs, distinguished from cells of macrophage series, it was necessary to spot well-defined criteria of identification ECs by which it was possible to distinguish them from cells of macrophage series, and on which it would be possible to find cells, from which are differentiated ECs. With the purpose of development of a complex scientifically justified of cytomorphological criteria of identification low-differentiate forms of ECs in culture in a following stage of operation the complex comparative examination of cytomorphology of Mphs, phagocytized of GZ, and mature ECs, which formatived clusters in vitro after a preliminary stimulation by a Freund's complete adjuvant (containing micobacterium of a tuberculosis - *M. tuberculosis*, killed by heat) in vivo (look previous division chapter) was conducted. For detection of cells, which could be surveyed as cells precursors of ECs, the legible morphological criteria of their identification were necessary. It was represented important quantitatively to estimate self descriptiveness separate cytomorphological and morphofunctional features of indications ECs, and also their combinations as criteria of identification of the different transition forms of ECs in culture. For the solution of this problem the multivariate cluster analysis and random principles of process of a discernment of objects utilised. The quantities of absolute probability of a discernment ECs in mixed culture Mphs and ECs on quantitative parameters calculated on the basis of multivariate cluster analysis of mixed sampling from Mphs, possessing of phagocytic activity were retrieved, and ECs, which accessories to epithelioid phenotype did not call doubt. The following absolute probabilities of a discernment of ECs in mixed cultures Mphs and ECs on quantitative parameters are fixed (Arkhipov, 2001a): maximal diameter of a nuclei ( $P = 0,95$ ), area of a cell ( $P = 0,73$ ), diameter of a nucleolus ( $P = 0,76$ ), nucleus-cytoplasm relations ( $P = 0,52$ ); amount of ruffles ( $P = 0,53$ ), amount of filopodiums ( $P = 0,60$ ); amount of lamellopodies ( $P = 0,55$ ). The padding measure of identification ECs, representing conditional probabilities of a

discernment ECs to quality indications that is probability of identification rated at performance of additional: spreading cell has the trapezoidal shape ( $P = 0,86$ ); the ellipse shape of a nuclei ( $P = 0,87$ ); uniform "mesh" allocation of a bazihromatin in nuclei, with a dominance of small lumps on a rim of nuclei and with "expressiveness" of an euchromatin ( $P = 0,93$ ); a rounded profile of a regional zones of cytoplasm ( $P = 0,95$ ); availability of the expressed cones of body growth ( $P = 0,99$ ); undermembrane fascicles of actinic filaments posed mainly in one direction ( $P = 0,99$ ); microvillis scarle ( $P = 0,54$ ) uniformly distributed. The multivariate cluster analysis based on the registration of three different measure of identification (with high probability of a discernment ECs) has shown that usage of several criteria of identification allows to identify all ECs in cultures Mphs with probability to equal unity, coming nearer to quantity. It is established that the criteria of identification of ECs, designed at examination ECs, formatived in cultures of the PCs, stimulate by Freund's complete adjuvant, well "work" in an estimation of cytomorphological measure in the relation of ECs, chosen from granulomas, induced hypodermic introduction by Freund's complete adjuvant or introduction BCG, and explantated in cultures in vitro. It is exhibited that cytomorphological feature of ECs, formatived in cultures of PCs after their "stimulation" in vivo (intraperitoneal introduction 50 % of emulsions Freund's complete adjuvant, prepared in physiological saline solution), and ECs, formed in granulomas, induced adjuvant or BCG, coincide on the majority of estimated parameters (shape of spreading cell, sizes and shape of nuclei, features of a structure of a nuclear chromatin, features of a structure of a regional band spreading cell, features of architectonics of actinic cytoskeleton, and also series of other cell-like performances). For more precise detection of all transition forms of ECs, and also low-differentiate forms cells-precursors, alongside with an estimation of phagocytic activity, it was necessary to explore some other parameters of their manifestation of their function activity. The estimation of ability of ECs was conducted to reduce nitroblue tetrazolium (NBT) to formazan, accumulate acridine orange (AO) and neutral red (NR) in lysosomes, to be coloured by a vital stain Janus green (intravital colouring on a mitochondrion). Besides these the estimation of peroxidase activity of ECs was conducted. As for all ECs of a nuclei not enough "heterochromatin" contained and in the morphological schedule were described as a nuclei with dominance "euchromatin" (as the testimony of high function activity of nuclei) the immunocytochemical colouring of nuclei on expression in nuclei the histone protein H1 was conducted. As a result of these examinations it is fixed following. ECs as well as Mphs were capable to reduce NBT, but the character of allocation of formazan in cytoplasm of Mphs and ECs essentially differed. For Mphs the accumulation by the way large heteromorphic lumps in a perinuclear band of cytoplasm was characteristic. In cytoplasm of ECs finely divided inserts formazan were proportioned rather uniformly. Moreover, through a polarization microscope was fixed that the shaping lumps of formazan can happen and on an exterior surface of a cell-like membrane ECs that testifies to availability for ECs of the mechanism of exocellular production of the free forms of oxygenium. The character of colouring ECs and Mphs by a fluorescent stain of AO and vital stain by NR also differed. The lysosomal structures of Mphs, accumulating lizosomotropny stain NR, were rather heterogeneous on the sizes, AO in ECs was stored in small lysosomes (or secretory granulas?) comparable on quantity. The mitochondrions, painted by Janus green, in ECs were of the mainly prolate shape and the polarizations of a cell are oriented lengthwise axis that probably was stipulated by a locating

of fascicles of undermembrane actinic filaments (stresses - fibers), a defining direction "polarization" of ECs. ECs had no peroxidase activity. The different degree of peroxidase activity was detected for 17,5 % of cells referred on cytomorphological features to monocytes / Mphs. Nuclei of all ECs in a different degree express a histone H1 that testified to different genetic activity of nuclei of ECs. As a result of comparative cytomorphologic analysis Mphs and all transition forms of ECs, formatived in culture, and also designed on its basis of criteria of identification of ECs in culture following cytomorphologic indications, describing low-differentiate cells, referred to cells-precursors of ECs, were established. Pre-ECs are cells about a diameter 15-23 microns (in spreading state), having a major round or oval excentrically posed nuclei (diameter 12-16 microns) with a major nucleolus, nucleus-cytoplasm relation  $> 1$ ; the surface of nuclei flattened and has no penetrating impressions; chromatin of nuclei is fine-structural, mesh, having characteristic drawing of alternating strips of an enlightenment. Cytoplasm is weak basophilic. In cytoplasm of some cells in small quantity thin eosinophilic and azurophilic graininess are taped. Amounts of lysosomes in cells is major. A topography of a surface pre-ECs by places plaited with small quantity of microvillis. Peroxidase activity is missed. The colouring of cytoplasm in the NBT-test is well expressed. Expression of a H1-histone is brightly expressed (Arkhipov, 1997, 2001a, 2004). After explantation in cultures of cells with phenotype pre-ECs in 1-2 hours after an attachment gain characteristic for ECs the triangular, trapezoidal or polygon shape with cones of growth (Fig. 5).

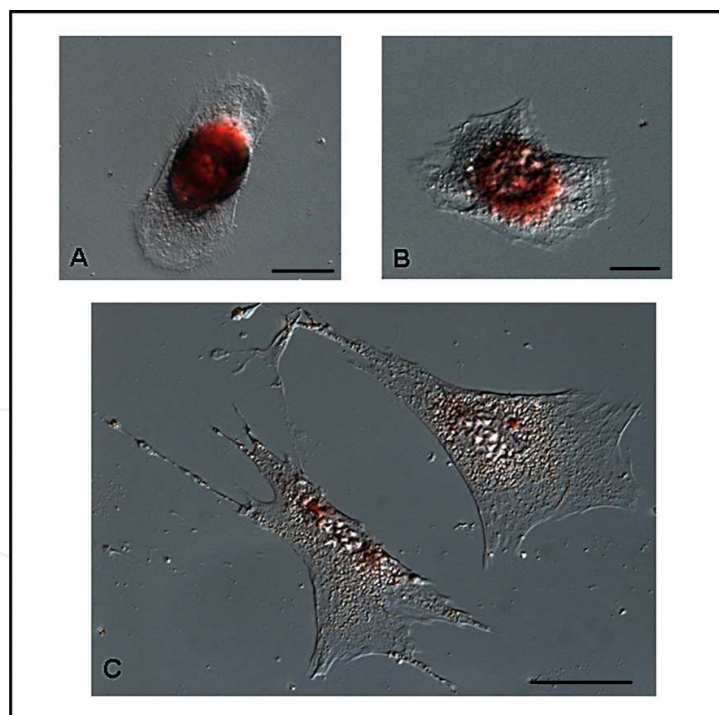


Fig. 5. Culture of PCs of mice of line BALB/c, beforehand stimulated in vivo (7 days prior to an explantation of cells) by Freund's complete adjuvant (intraperitoneal introduction 50 % of emulsion in physiological saline solution). A - pre-EC, B - "«transition" form of pre-EC, C - "«juvenile" form of ECs. Vital colouring neutral red (accumulation of a stain in lysosomes), additional contrasting of cells by a method of differential interferential contrast. Scale bar: 10  $\mu$ m.

To be sure that the fixed measure of identification pre-ECs are reliable and substantially work, it was necessary to conduct a series of examinations on a statistical validity and proof of a position that everything the cells, detected with phenotype, marked as "«pre-ECs", are differentiated in cells of epithelioid type.

6. Statistical validity and confirmation of existence of a specialized line of cells-precursor of epithelioid cells (pre-ECs)

The statistical validity of existence of an epithelioid-cell-like line in an organism - population of cells, committed to formation of ECs in norm that in the absence of inflammatory response or any pathological process in an organism was conducted. In the fig. 6 the data on the dynamics of accumulation in cultures of all transition forms of ECs, represented in cultivating of PCs of intact mice, liberated from lymphocytes and other nonadhered cells. By cytomorphological study of such cell-like cultures for various periods of cultivation determined that the amounts of cells, identified in the first hour of cultivation as a poorly differentiated pre-ECs, reliably does not differ from amounts of mature ECs, detected to 7 days of cultivating. As it is visible from the fig. 6 the quantity of all transition forms of ECs in control (intact mice) on the 7-th day of cultivation practically corresponds to starting quantity of pre-ECs in culture, which is registered in the first hours of cultivation. Moreover, it is exhibited that in cultures of cells of intact mice for 14 day cultivation, the total unities of epithelioid-cell-like forming - separately spaced apart of ECs and epithelioid-cell-like clusters, formation which occurs as a result of cell division, it is also statistically identical.

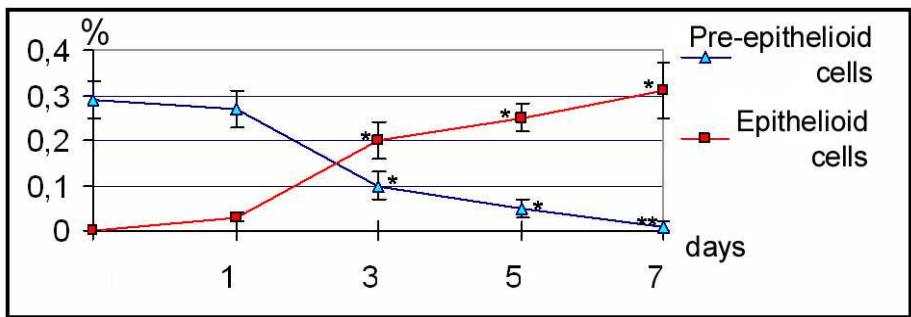


Fig. 6. The dynamics of accumulation of all transition forms of ECs cells in culture of PCs of intact mice of line BALB/c. The results are presented in the form  $\bar{x} \pm s_x$  (%), where  $\bar{x}$  - arithmetic mean (average),  $s_x$  - standard error of the mean. Significant differences between groups was assessed using the nonparametric Wilcoxon-Mann-Whitney test (\* $p < 0,05$ ; \*\*  $p < 0,01$ ).

In the Fig. 7 shows the dynamics of accumulation of all transition forms of Ecs that was registered in examination of PCs of mice, whom at least 7 days prior to explantation of cells in vitro were injected intraperitoneally Freund's complete adjuvant. The intraperitoneal introduction of Freund's complete adjuvant results in augmentation of amounts of cells-precursors of ECs in 5 times in comparison with their quantities in the "control" (intact mice) and reaches in separate cultures to more 1,0 %. "Disappearance" pre-ECs (bound from them differentiation and transformation to more mature forms) in cultures of cells, stimulated by Freund's complete adjuvant, is accompanied in the beginning by augmentation of quantity



of the "juvenile" forms of ECs for 5 day of cultivation. With further cultivation the quantity of the "juvenile" forms of ECs is reduced and the number of mature ECs, including ECs, which are in phases of growth and reproduction, increases exponentially. The phase of a proliferation is characterized by augmentation in culture of quantity of ECs with nuclei having indications of endomitosis (with characteristic "patterns" with more or less discernible "contours" of chromosomes), two-nuclear ECs and clusters of ECs.

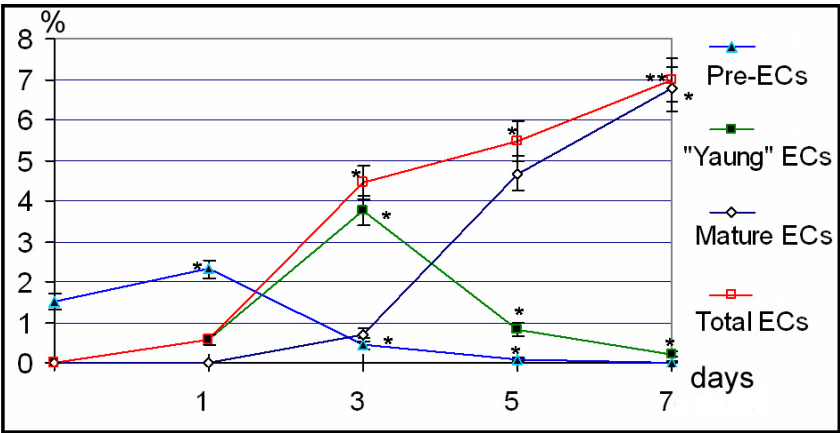


Fig. 7. The dynamics of accumulation of the different transition forms of ECs in culture of PCs of line BALB/c, stimulated in vivo by Freund's complete adjuvant. Transsudate of PCs, obtained in 7 days after intraperitoneal injection of adjuvant. On an axis of ordinates - quantity of ECs (in % from all cells in culture). The results are presented in the form  $\bar{x} \pm s_x$  (%), where  $\bar{x}$  - arithmetic mean (average),  $s_x$  - standard error of the mean. Significant differences between groups was assessed using the nonparametric Wilcoxon-Mann-Whitney test (\* $p < 0,05$ ; \*\*  $p < 0,01$ ).

In "control" (not stimulated cell cultures of intact mice) only simple ECs transferred in a stage of a proliferation to 14 days of cultivation, forming simple clusters of 3-5 ECs. When cultured PCs, activated with Freund's complete adjuvant, marked stimulation (acceleration) of all the processes that determine the differentiation of pre-ECs into ECs - from the beginning of the transformation pre-ECs in ECs to phase of reproduction with the formation of large clusters and 2-layered epithelioid-cell layers. At the same time noted stimulation of Mphs apoptosis, taking place in immediate proximity from ECs, with formation numerous apoptotic bodies, which are likely to use EC as an additional plastic material in active growth and proliferation of ECs (see Fig. 3). The obtained data have allowed to make following findings: 1) in a population of PCs there is a subpopulation low-differentiated cells, morphologically distinguished from cells macrophage line (monocytes / Mphs), being cells - are predecessors of all cells of epithelioid type, 2) ECs are differentiated only from population of pre-ECs, committed to a direction of epithelioid-cell-like cytomorphogenesis, 3) at long-lived cultivation and stimulation of PCs of mice the part "mature" ECs is capable to transfer in a phase of a proliferation, forming colonies, consisting of fitting closely to each other cells - epithelioid-cell-like clusters and epithelioid-cell-like layers. Actually obtained data have allowed to conclude that the process of epithelioid-cell-like forming in vitro limited by a separate single-cell epithelioid cell line, that is histogenetic non-macrophage cell line.

## 7. The rating of a contents of cells-precursor of epithelioid cells in a blood and bone marrow (pre-pre-ECs)

Based on already set criteria of identification of pre-ECs, obtained in the study of cultures of PCs, represented major practical and theoretical interest installation the rating of a contents of cells-precursor of ECs in a blood and bone marrow, conditionally marked as "pre-pre-ECs". By the method of intravital observation in microchambers fixed that within 3 hours after beginning of cultivation of blood monocytes of mice some large-scale "plasmacyte-like" monocytes gain at spreading the shape, characteristic for differentiated pre-ECs - triangular or trapezoidal with characteristic growth cones (to look fig. 5). As against the majority of typical monocytes (being the precursors of Mphs) with characteristic for Mphs "bean-shaped" or "blade-shaped" nuclei, polymorphic type of spreading (distinguished major variety of the shapes) and possessing of phagocytic activity (concerning to microspheres of polystyrene latex, granulas of a zymosan and micobacteria BCG), large-scale "plasmacyte-like" monocytes named "Epitheliocytoblast" did not phagocytosed (Arkhipov, 1997, 1999a, 1999b). With further cultivation (about 1-2 days), "plasmacyte-like" monocytes were polarized and gained a view, characteristic for "juvenile" ECs. The series of experiments for a statistical validity of existence of an epithelioid-cell-like line in a blood - the population of cells, committed to formation of ECs, was conducted. The comparison of estimations of the number of cells - precursors of ECs, obtained on the basis of the analysis of short-term cultures of leucocytes (3 hours), and also analysis of cultures of leucocytes through 2 days after the start of cultivation, was conducted. The study showed that amounts of pre-ECs for mice of line BALB/c (in recalculation on all leucocytes), detected by these methods, and the amounts of the "juvenile" forms of ECs is determined by one quantity - 0,01-0,03 %. It is established that among all cells of a bone marrow there are cells, which on series of morphological indications (sizes of a cells, sizes and shape of nuclei, features of a structure of a chromatin etc.) and on availability of particular colouring in transiting and reflected light, it is possible to refer to cells - precursors of ECs. Their quantity laid within the limits 0,005-0,01 %. For a number of cytomorphological features (after colouring by azure and eosine) unspread cells of this type have likeness to major reticular cells of a bone marrow, which rank as cells of a reticular stroma of a bone marrow. Thus, as a result of al conducted studies was found that quantity of a pool pre-ECs in a peritoneal cavity in norm for mice BALB/c reaches 0,05-0,15%, among leucocytes of a blood - 0,01-0,03%, bone marrow - 0,005-0,01 %.

## 8. The rating of speed of differentiation of pre-pre-ECs (from blood and bone marrow) and pre-ECs (from peritoneal cavity) at different differential stimulus in vitro

To one of indexes of maturity of pre-ECs it was possible to refer ability them to differentiation. The estimation of potencies of pre-pre-ECs and pre-ECs to differentiation into ECs at adding in medium the stimulants of cell differentiation was conducted. The stimulation of differentiation of pre-pre-ECs and pre-ECs in cultures realized by an incubation in medium RPMI-1640, containing Potassium Orotate (PO; 5 µg/ml) and Dimethyl sulfoxide (DMSO; 0,005 % in the final dilution). The effects estimated at count of in each culture  $10^4$  PCs,  $10^5$  leucocytes of a blood and  $10^5$  cells of a bone marrow (5 hours after the start of cultivation). At cultivation of PCs within 5 hours the greatest amounts of

the "juvenile" forms of ECs is detected only in those cultures, to which imported PO and DMSO. It is exhibited that the acceleration of processes of differentiation of pre-ECs into cells of an epithelioid type is higher in cultures of PCs received from animals stimulated by Freund's complete adjuvant or BCG. The similar patterns of cytomorphogenesis of ECs are detected at examination of effect of a stimulation of differentiation of pre-ECs (or "pre-pre-ECs") of a blood and cells of a bone marrow (Arkhipov, 2001b). It was found that under selected requirements of cultivation, potency of cells of a bone marrow, leucocytes of a blood and PCs, referred by cytomorphological measure to pre-ECs, to differentiation into ECs are various. They have minimal for pre-ECs of bone marrow, but are high enough for pre-ECs of a blood. It is exhibited that the number of active peritoneal pre-ECs, capable of rapid starting of the mechanisms of differentiation of pre-ECs, defining ECs-forming, exceeds the number of active pre-ECs in the blood. Apparently, that at usage of any other conditions or methods of cultivation of cells of bone marrow (at usage of the padding growth factors, any more effective stimulators of cell differentiation) more optimal requirements, promoting for pre-pre-ECs of bone marrow to differentiation into ECs in vitro can be found.

### **9. The rating changes of a pool of pre-pre-ECs in a blood in the development of an experimental chronic inflammation in mice**

Based on the obtained data it was possible to assume that the number of pre-pre-ECs in the blood may vary depending on the "stage" or "outcome" of the pathological process, in some way definitely reflecting the dynamics of development or involution of epithelioid-cell-like granulomas in an organism. To answer this question has been used a model of granulomatous inflammation in the liver granulomas with involution, and the developed on its base model of chronic granulomatous inflammation in the liver with different outcomes (Arkhipov, Shkurupy, 1996). It was shown that changing the number of inputs pure zymosan granules (GZ) and the number of grain boundaries associated with acid fuchsin (GZF), you can change the dynamics of granuloma Mphs update, their differentiation and, consequently, to model different versions of morphogenesis of ECs granulomatous inflammation. It is shown that 7 days after intravenous injection of pellets of GZ and zymosan, chemically modified acidic fuchsin (GZF), develop in the liver macrophage granulomas, containing a small amount of ECs and lymphocytes. When using GZF the number of ECs-granulomas was slightly higher (by 25 %), than with using GZ. By 21-th days the results in the indicated experimental groups differed significantly. The number of granulomas induced by GZ was decreased to 4.0 in 10 fields of view, and induced by GZF practically no change, reaching a value of 63,7%. It is established that a pool of pre-ECs in blood changes in the development of granulomatous inflammation in some way reflect the dynamics of the development of granulomas and their involution. It was important to evaluate changes of pool progenitor cells of ECs in a typical epithelioid-cell granulomatous inflammation. To meet this challenge the model of chronic disseminated tuberculous inflammation was used. Granulomatous inflammation in various organs of mice of BALB/c induced by intraperitoneal injection of Mycobacterium Bovis BCG (1 mg/ animal). Estimate of the number of granulomas in liver was carried out on histological preparations, stained with hematoxylin and eosin. The number of pre-ECs in the blood was assessed in short-term cultures of leukocytes. It is shown that 30 days after intraperitoneal injection of BCG in the

liver developed typical epithelioid-cell granulomas. These granulomas are stored in the liver for several months. It was assessed the changes in the blood pool of pre-ECs at 1 and 2 months after BCG injection. It is shown that in mice at 1 month after injection of BCG was an increase in the circulating pool of progenitor cells of ECs in the blood of almost 3,5 times, compared with the control (intact animals). The number of granulomas in histological preparations in 10 fields of view was 7,2. In 2 months after the induction of granulomatous process the amounts of granulomas in the liver decreased by 1,7 times. In the proportional reduction of pool pre-ECs in blood is noted (1,8-fold, compared to that at 1 month after the intraperitoneal injection of BCG). Correlation analysis of the data showed that between the number of epithelioid-cell granulomas in the liver of mice and the number of epithelioid-cell precursors (pre-pre-EC) in the blood is a correlation ( $r = 0,96$ ,  $P < 0,05$ ). Thus it is shown that the processes of morphogenesis of epithelioid-cell granulomas may greatly determine by inflow to the site of granulomatous inflammation progenitor cells of ECs.

#### **10. The rating of a circulating pool of pre-ECs in mice of different genetic lines of different predisposition to development of a tubercular infection**

It is known that immunological failure of those or other links of immunity, contributing to some inflammatory and infectious diseases, including tuberculosis, are genetically stipulated and are interlinked to failure of the particular immunological factors (Cardona, 2004; Shkurupy, 2007). It was expressed the guess that the quantity of pre-ECs also can be genetically determined. For study of a genetic condition of the control of epithelioid-cell-like cytomorphogenesis the following lines of mice were selected: BALB/c, C57BL/6, CBA, DBA. According to the data of the scientific literature these lines of mice at pairwise comparison can be referred to "opposite reacting lines", concerning formation of epithelioid-cell-like granulomas by infection of the same agents - micobacteria of a vaccine BCG or virulent forms of micobacteria of a tuberculosis. For all lines of mice estimated amounts of cells-precursors of ECs in abdominal cavity, committed in an epithelioid-cell-like direction of differentiation - pre-ECs. The indicated parameters estimated for intact animal, and also after an induction in mice an inflammation in abdominal cavity by introduction of an emulsion of a Freund's complete adjuvant. It is exhibited that per quantity of a starting pool of pre-ECs the explored lines of mice can be arranged on incremental in following series: DBA (0,02-0,09 %;  $0,07 \pm 0,01$  %), BALB/c (0,05-0,15 %;  $0,11 \pm 0,01$  %), CBA (0,07-0,19 %;  $0,15 \pm 0,02$  %), C57BL/6 (0,11-0,27 %;  $0,24 \pm 0,03$  %). At an induction of a chronic inflammation in abdominal cavity by introduction of emulsion of a Freund's complete adjuvant this tendency is maintained. However, thus the interlinear differences, estimated by quantity of induced cells of an epithelioid type, essentially will increase. If for mice of a line DBA, the amounts of ECs-precursors of ECs type at induction of an inflammation in abdominal cavity will increase only in 1,5 times that for mice BALB/c, CBA, C57BL/6 - accordingly in 3,9, 4,0 and 6,7 times. The obtained results allow to make a deduction about existence of a genetic determinancy of a datum level of an epithelioid-cell-like reactivity. They specify also that the morphogenesis of epithelioid-cell-like granulomas in tuberculosis can be determined by the different starting genetically determined level of a pool pre-ECs, inflow pre-ECs in the center of an inflammation, and also intensity of processes them differentiation into ECs.



## 11. The conclusion about necessity of revising of the old concept of a origin of epithelioid cells of cells of system of mononuclear phagocytes

The analysis of the scientific literature touching problems of a nature and cytomorphogenesis of ECs, and also analysis of the experimental data, introduced in the present chapter, allows to raise the question about revising the resisted representations about a parentage ECs from Mphs. Tuberculosis - very dangerous, serious and artful disease. And we can not neglect even slightest chance in improving comprehension of its etiology, pathogeny and morphogenesis of epithelioid - cell-like granulomas (or tuberculomas), which are surveyed by the majority of research peoples as structures, which function is isolation of an organism from the centers of an infection that is "function" of protection of an organism from a further dissimulation of micobacteria of a tuberculosis in an organism. From positions of the concept of a parentage of ECs from Mphs, to ECs the role of enough "passive" cells, namely modified Mphs with the "truncated" functions (decay the phagocytic activity etc.) is tapped. Thus it is supposed that early or late in tubercular process Mphs in formatived granulomas are transformed into ECs, which function till now is not spotted. We attempt to present a histogenesis of ECs and morphogenesis of tubercular granulomas process completely on new, from positions of a parentage from specialized cells precursors of ECs. In a particular stage of forming of granulomas from Mphs, the germ of pre-pre-ECs in bone marrow is stimulated, the pool of pre-pre-ECs in a blood will increase, they arrive in the center of granulomatous inflammation, which structural basis are Mphs, as well as the different subpopulations of lymphocytes, dendritic cells, and also coming monocytes (precursors Mphs). Pre-ECs are differentiated into ECs, and the process of augmentation of a pool ECs in granulomas happens both for the score of differentiation of pre-ECs, and at the expense of proliferative activity of ECs (division of cells as a result of an endomitosis or amitosis). The genetically determined reduce content of pre-ECs in an organism will give that in tubercular granulomas will dominate Mphs, formative macrophage granulomas (with huge multinuclear cells of foreign bodies), possessing to reduce "abjoint" potential. In the complete absence in the circulation system pre-ECs will be formed granulomas that do not contain ECs. If to go in our reasonings is farther it is possible to assume that ECs have any high special-purpose functions, which are not solved yet, and which allow these cells at prompt enough ascending of their pool in the center of contamination by micobacteria of a tuberculosis and prompt organization in granulomatous epithelioid-cell-like frames, to handicap with a dissimulation not only tubercular micobacteria from the centers of a tubercular lesions, but to fulfill «functions of abjoint» in other granulomatous diseases of a infectious and noninfectious etiology. The comparative analysis of cytomorphologic features of different types ECs, formatived in cultures in vitro, in a context of modern representations about differentiation of mesenchymal stem cells in different histogenetic directions cell-like differentiation, allow in a new fashion to survey the concept of a histogenesis of ECs from cells-precursors, committed in an epithelioid-cell-like direction of differentiation. It is possible that some epithelioid-like cells of the forms of cells, formatived in the centers of tubercular granulomatous inflammation (for example, " fibroblast-like" shape of ECs) can be offsprings of mesenchymal stem cells. The clearing up of the earliest stages of a histogenesis of ECs will allow to answer a problem not only about biological substance of ECs, formatived at different chronic granulomatous processes, but also more

precisely to spot their functionality in an organism in tubercular pathological process. The original positions of the new concept of a parentage and differentiation of ECs in chronic inflammatory granulomatous processes, including in a tuberculosis, schematically represented in fig. 8. For matching in the same scheme the conventional concept of a histogenesis of ECs represented.

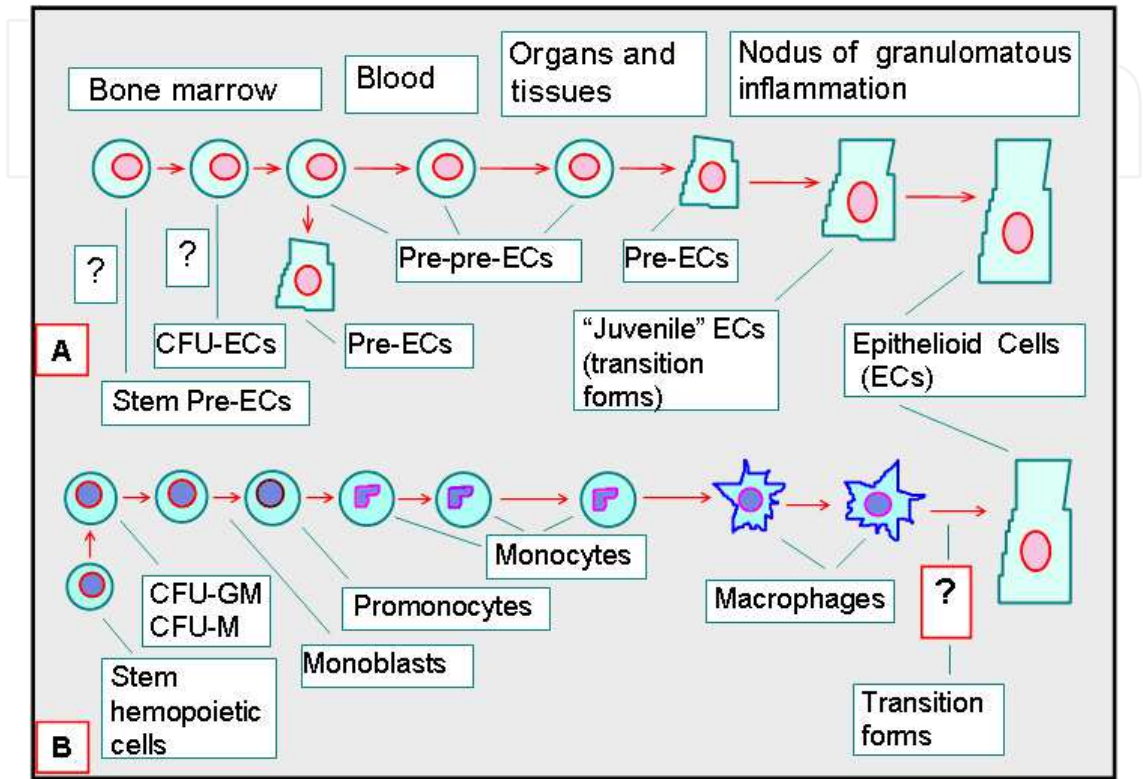


Fig. 8. The scheme of a histogenesis of ECs, constructed on the basis of the results of own examinations (A), in matching with the conventional scheme of epithelioid-cell-like cytomorphogenesis in chronic inflammatory granulomatous processes (B).

**12. The algorithm of further researches of epithelioid cells and the rating that can give new knowledge of epithelioid-cell histogenesis in comprehension of a morphogenesis of the tubercular process, in improvement of diagnostics of a tuberculosis, development of methods of its treatment and prognosis of development**

Now, unfortunately, we yet do not allocate precise enough immunophenotypic or cytochemical markers of ECs, permitting to correlate data, received in examinations of ECs with the help of morphological methods and on cultures of cells. Therefore, to one of the proximate tasks in examinations of ECs it is necessary to refer searching high-specific markers (clusters of differentiation, cytoskeleton proteins, receptors, enzymes), characteristic only for ECs and pre-ECs. Thus during these examinations any would be clarified «specific or specialized» functions of ECs, which they fulfill in the center of tubercular granulomatous inflammation. In my opinion, it is very complicated to calculate for major progress in the struggle against tuberculosis and in developments of new agents for treatment of tuberculosis, not having faithful representations about a histogenesis of ECs

and about functions, which they fulfill in tubercular granulomas. The new knowledge of a histogenesis of ECs from committed pre-ECs gives new comprehension of mechanisms underlying a morphogenesis of epithelioid-cell-like granulomas in tubercular process, and in long-range researching in this direction, can promote improving of diagnostics of a tuberculosis, help in development of new methods of treatment and prognosis of development of this disease. For example, the estimation of quantity of pre-ECs in a blood in the beginning of disease can specify on what “trajectories” there can be a process of formation of tubercular granulomas: macrophage, epithelioid-cell-like or mixed. The lack of pre-ECs in a blood can specify probable unfavorable development of granulomatous process with a probable prompt dissimulation of micobacteria of a tuberculosis in an organism. On the contrary, relatively large value of a pool of pre-ECs in blood and its ascending after infection can testify to probable more effective cupping of tubercular process as a result of prompt formation of epithelioid-cell-like granulomas. The same measure can be surveyed as an index of efficiency of a conducted immunotherapy and medicinal therapy of a tuberculosis as a whole. In conclusion follows to answer the question which will undoubtedly be raised. What does the new conception of origin and differentiation of epithelioid cells give? The new conception of epithelioid cells origin and differentiation - first of all, is a key to new understanding of concrete cell mechanisms that participate in forming of epithelioid cells granulomas and epithelioid cells-clusters in a tuberculosis, it is a key to correct understanding of pathogenesis and morphogenesis of the tuberculosis disease; probably it is the basis for elaborations of new diagnostic methods of the prognosis and new medical treatment methods of a tuberculosis. It would be desirable to hope that the results of experimental researches on study of histogenesis, cytomorphogenesis, morphofunctional potency of ECs, their role in pathogenesis and morphogenesis of the tubercular process, introduced in the present chapter, will not stay without attention and become a basis for further examinations in this direction.

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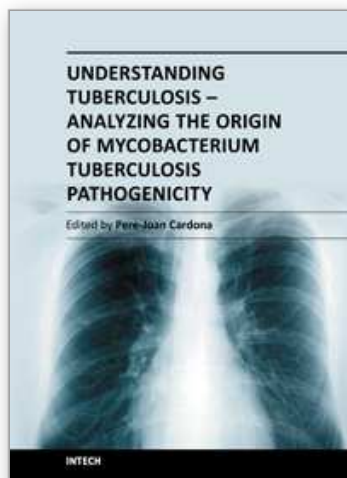
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## **Understanding Tuberculosis - Analyzing the Origin of Mycobacterium Tuberculosis Pathogenicity**

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Mycobacterium tuberculosis in an attempt to understand the extent to which the bacilli has adapted itself to the host and to its final target. On the other hand, there is a section in which other specialists discuss how to manipulate this immune response to obtain innovative prophylactic and therapeutic approaches to truncate the intimal co-evolution between Mycobacterium tuberculosis and the Homo sapiens.

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